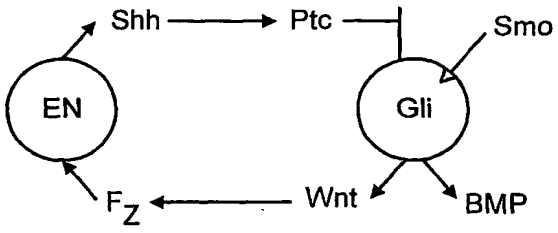

Shh
Ptc
Smo
EN
Gli
F_Z
Wnt
BMP

FIG. 1

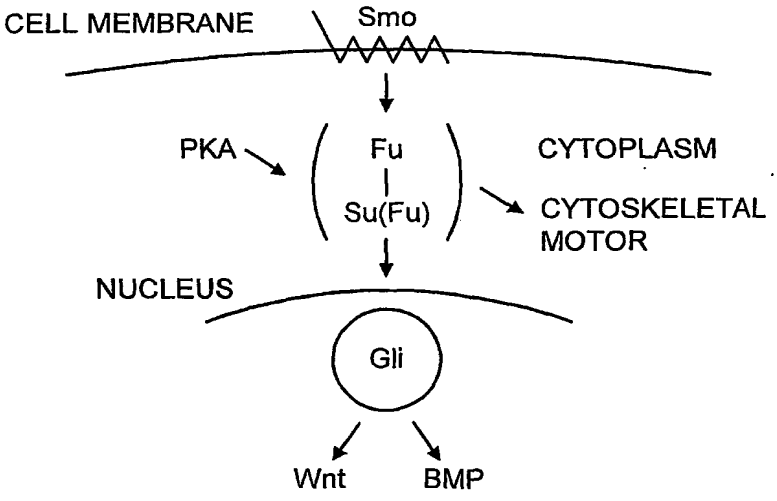

CELL MEMBRANE
Smo
PKA
Fu
Su(Fu)
CYTOPLASM
CYTOSKELETAL
MOTOR
NUCLEUS
Gli
Wnt
BMP

FIG. 2

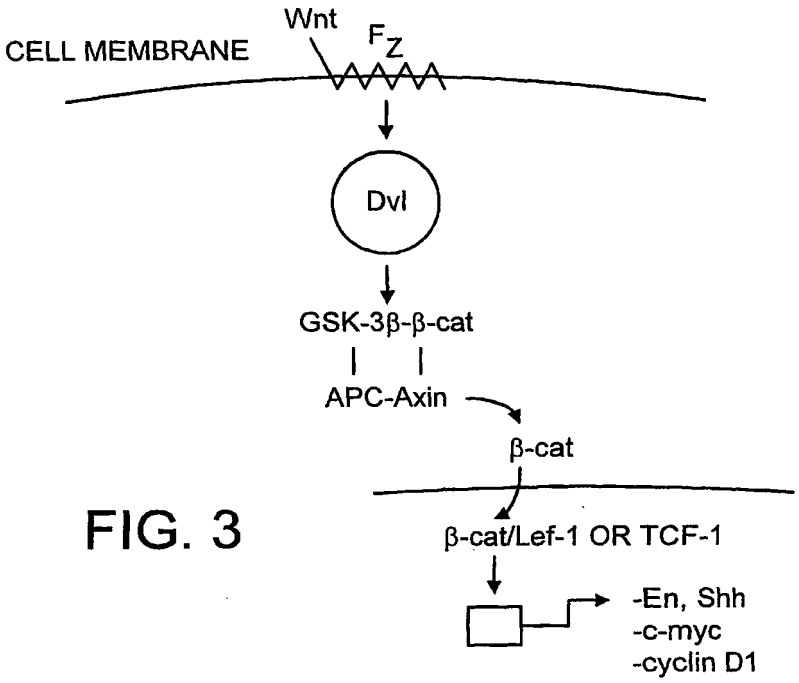

CELL MEMBRANE
Wnt
FZ
Dvl
GSK-3β-β-cat
APC-Axin
β-cat
β-cat/Lef-1 OR TCF-1
-En, Shh
-c-myc
-cyclin D1

FIG. 3

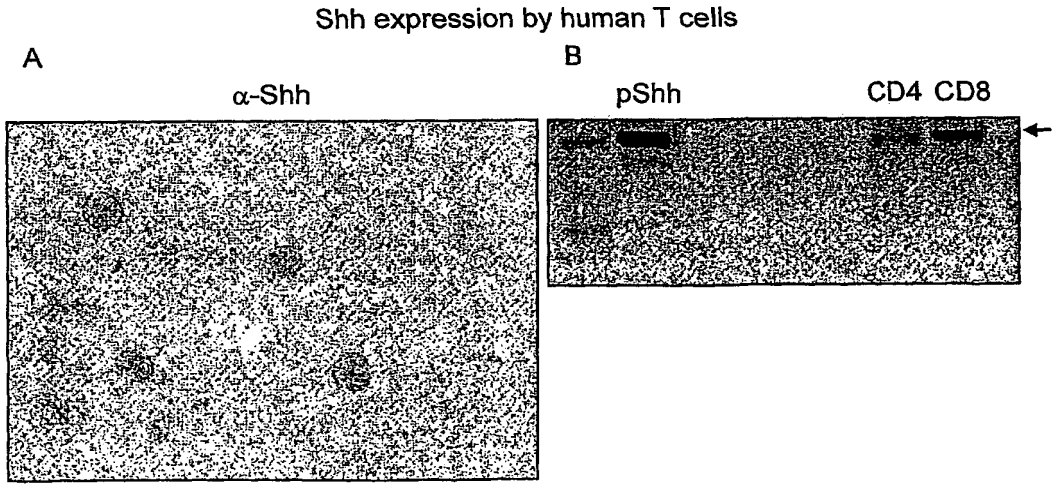
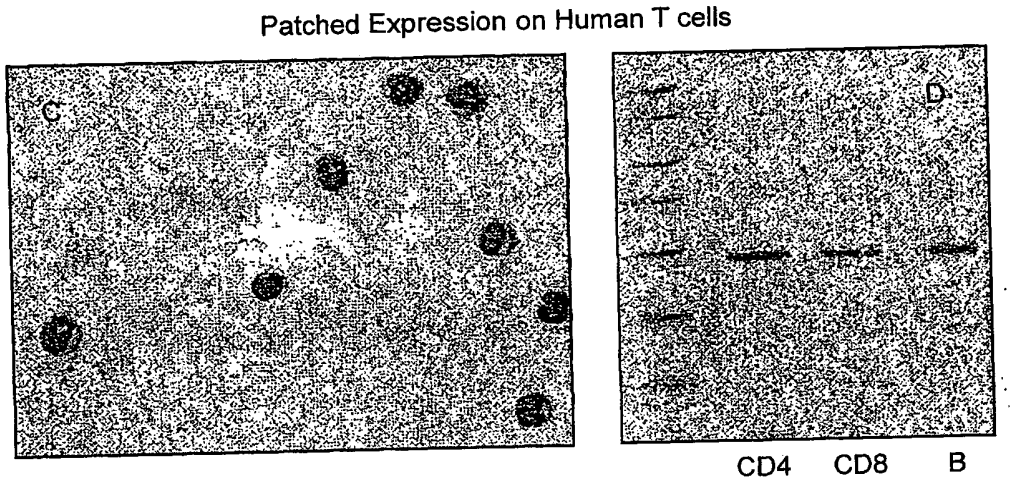

Shh expression by human T cells
A
B
α-Shh
pShh
CD4 CD8

FIG. 4


Patched Expression on Human T cells
C
D
CD4
CD8
B

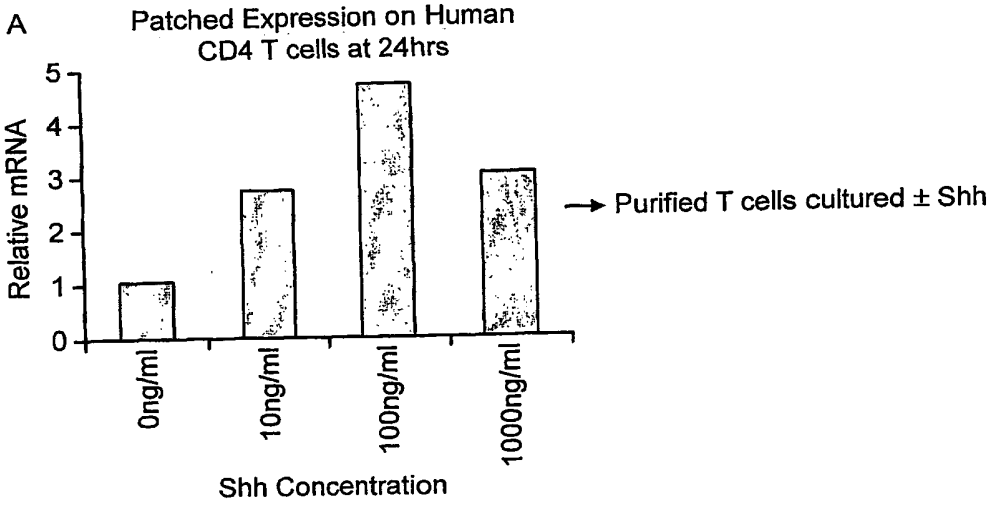

A
Patched Expression on Human CD4 T cells at 24hrs
Relative mRNA
5
4
3
2
1
0
0ng/ml
10ng/ml
100ng/ml
1000ng/ml
Shh Concentration
Purified T cells cultured ± Shh

FIG. 5

Expession of components of the Hedgehog pathway
in peripheral lymphoid tissues

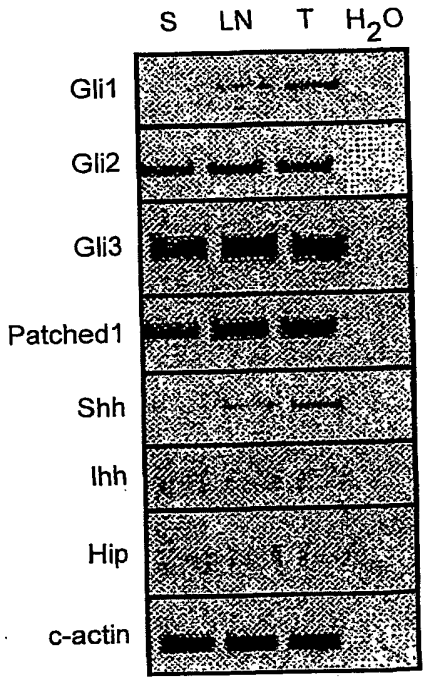


FIG. 6

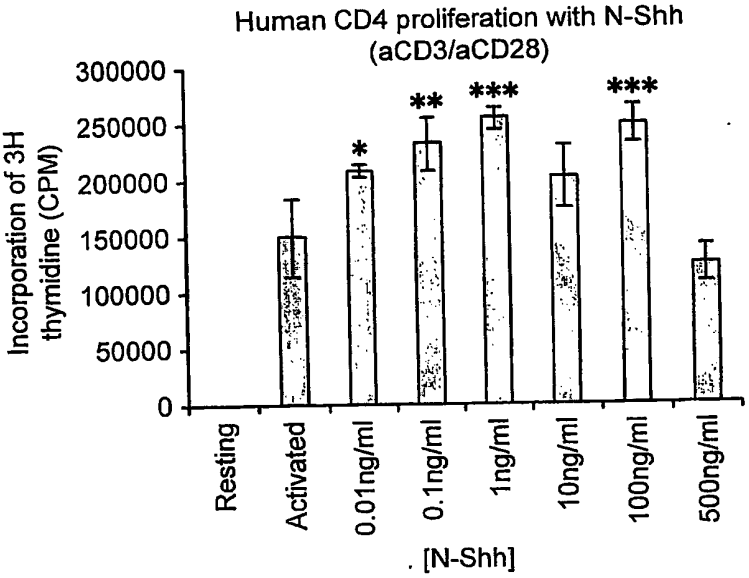

Human CD4 proliferation with N-Shh
(aCD3/aCD28)
Incorporation of 3H thymidine (CPM)
300000
250000
200000
150000
100000
50000
0
*
**
***
***
Resting
Activated
0.01ng/ml
0.1ng/ml
1ng/ml
10ng/ml
100ng/ml
500ng/ml
[N-Shh]

FIG. 7

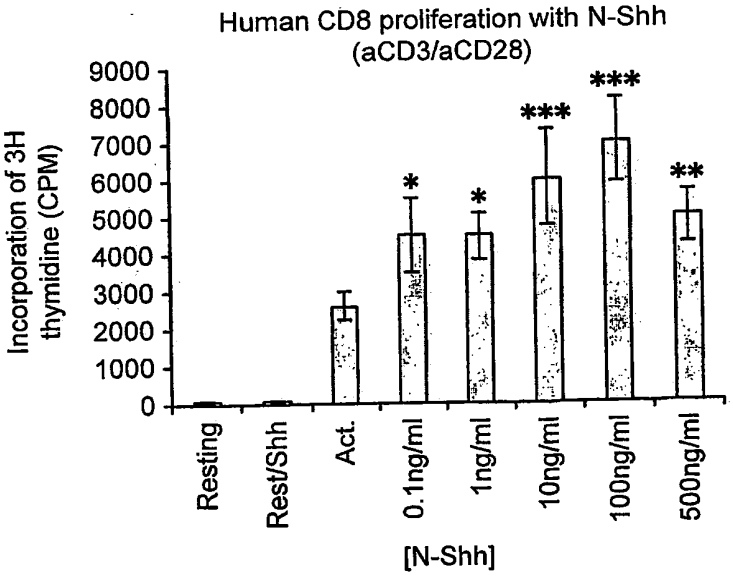
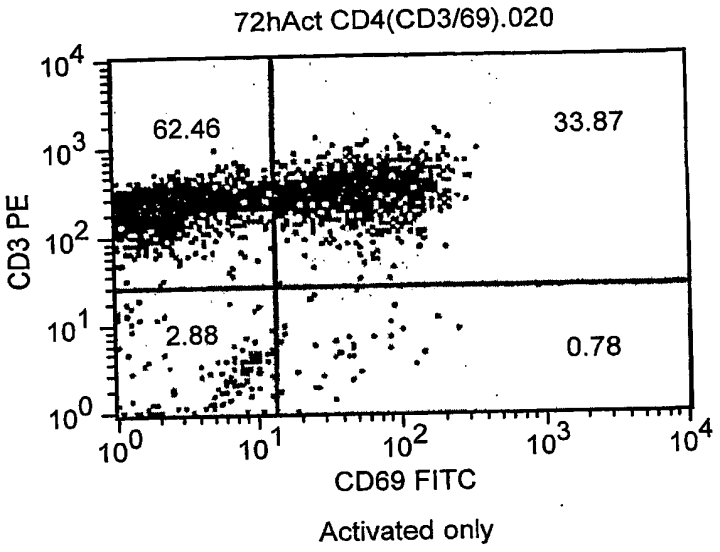

Human CD8 proliferation with N-Shh
(aCD3/aCD28)
Incorporation of 3H thymidine (CPM)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
*
*
***
***
**
Resting
Rest/Shh
Act.
0.1ng/ml
1ng/ml
10ng/ml
100ng/ml
500ng/ml
[N-Shh]

FIG. 8

Activated CD4s: CD3/CD69



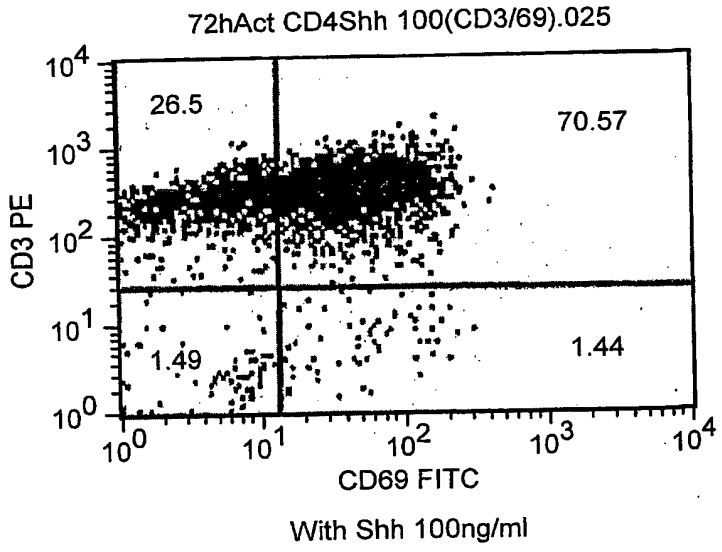
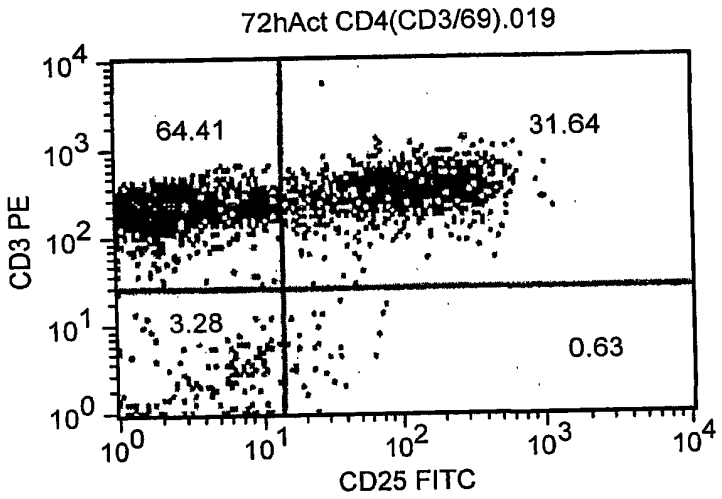


FIG. 9

Activated CD4s: CD3/CD25



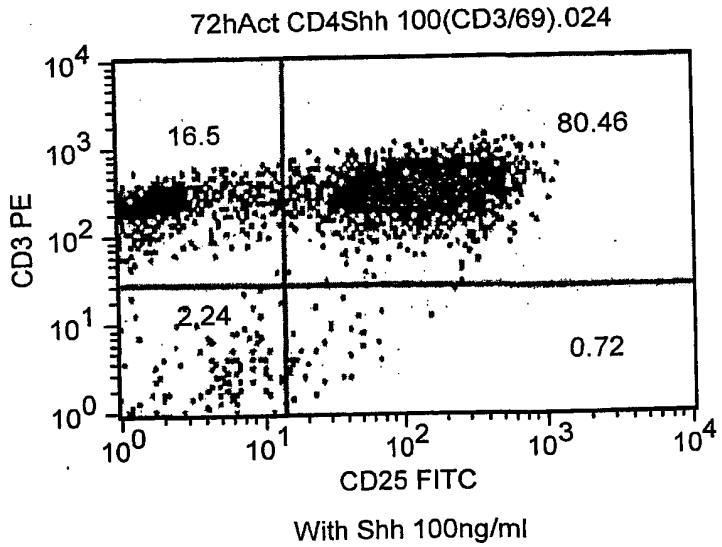


FIG. 10

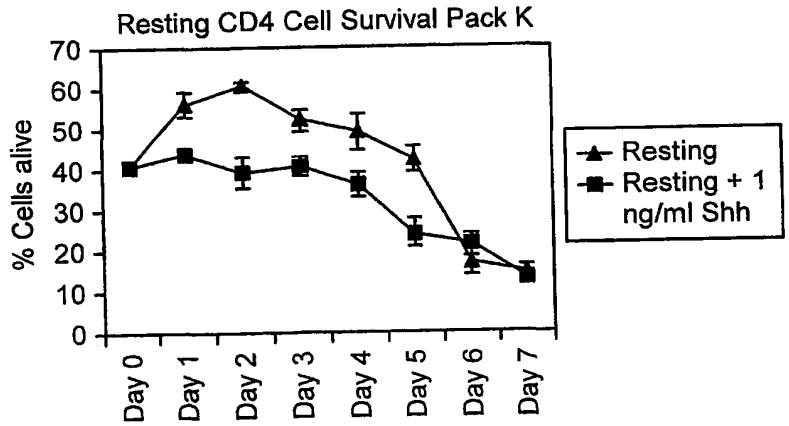

Resting CD4 Cell Survival Pack K
% Cells alive
70
60
50
40
30
20
10
0
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Resting
Resting + 1 ng/ml Shh

FIG. 11

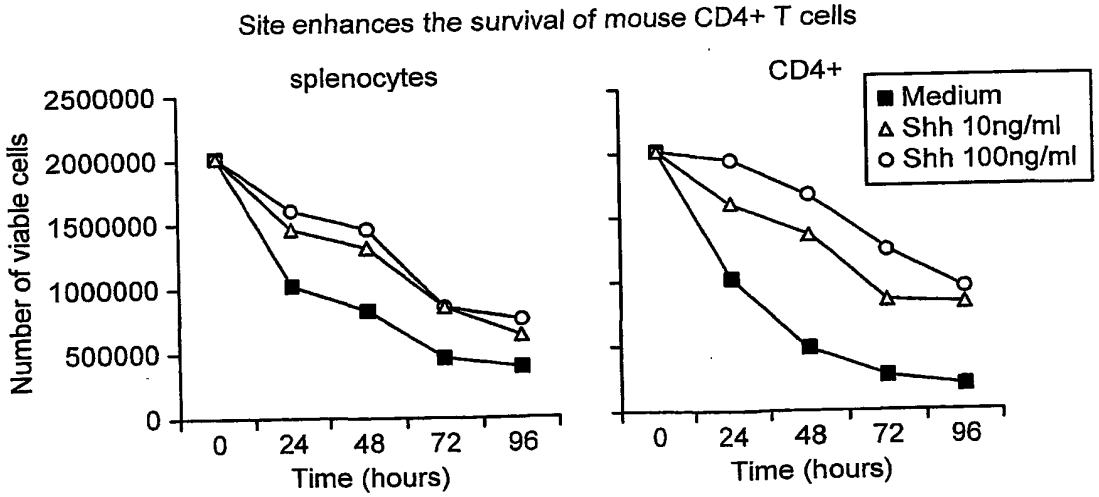

Site enhances the survival of mouse CD4+ T cells
splenocytes
CD4+
Number of viable cells
2500000
2000000
1500000
1000000
500000
0
0
24
48
72
96
Time (hours)
Medium
Shh 10ng/ml
Shh 100ng/ml

FIG. 12

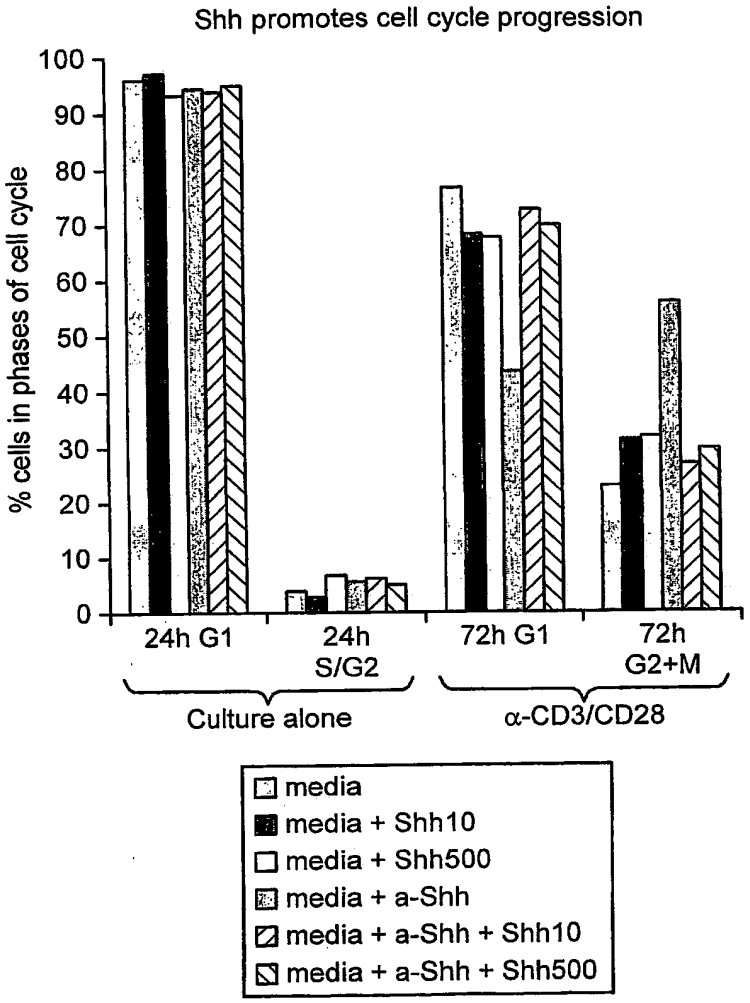

Shh promotes cell cycle progression
% cells in phases of cell cycle
100
90
80
70
60
50
40
30
20
10
0
24h G1
24h S/G2
72h G1
72h G2+M
Culture alone
α-CD3/CD28
media
media + Shh10
media + Shh500
media + a-Shh
media + a-Shh + Shh10
media + a-Shh + Shh500

FIG. 13

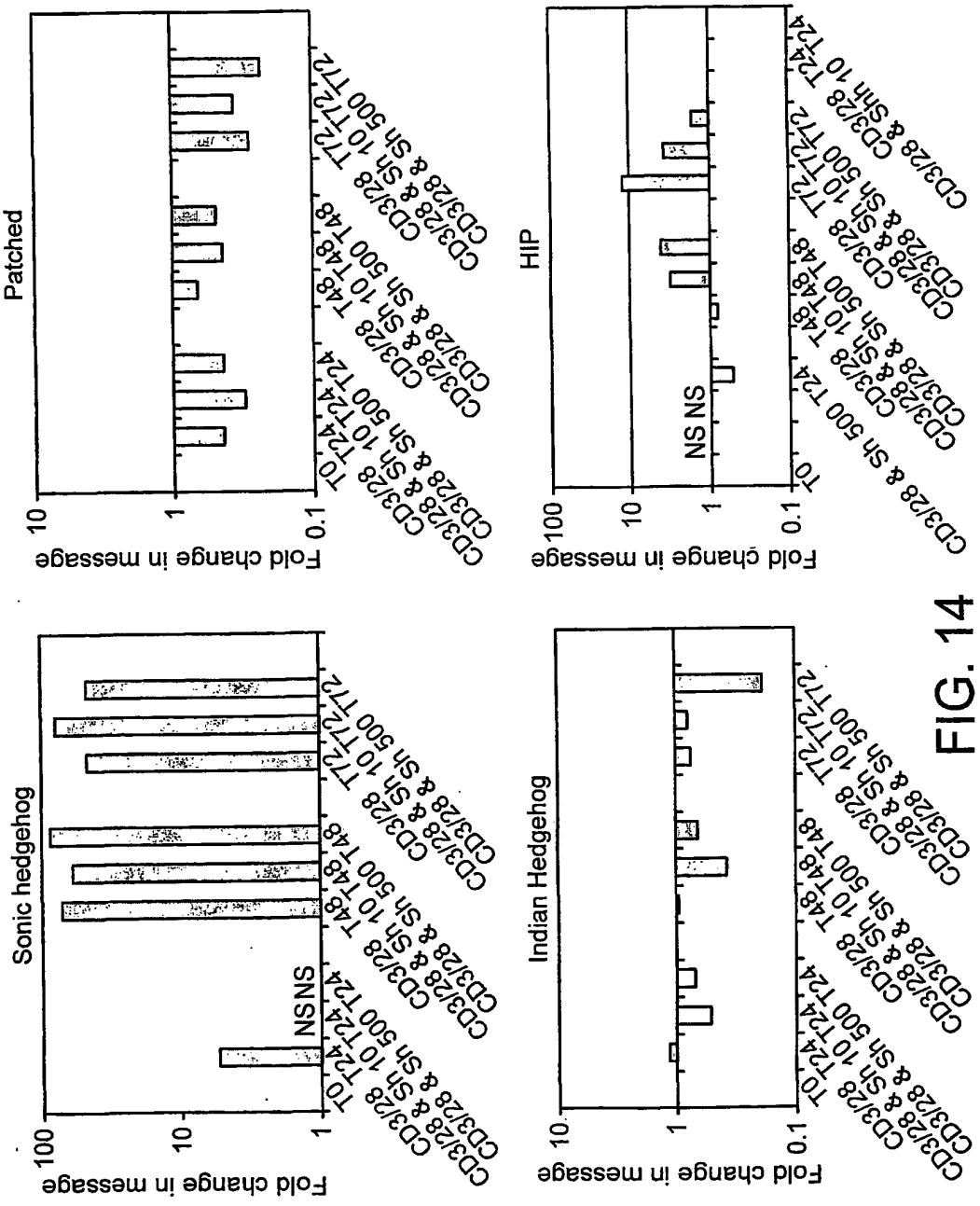

Patched
Fold change in message
10
1
0.1
T0
T24
10 T24
500 T24
T48
10 T48
500 T48
T72
10 T72
500 T72
CD3/28 & Sh
HIP
NS NS
Sonic hedgehog
Indian Hedgehog
100

FIG. 14

Induction of Shh on activated CD4+ T cells

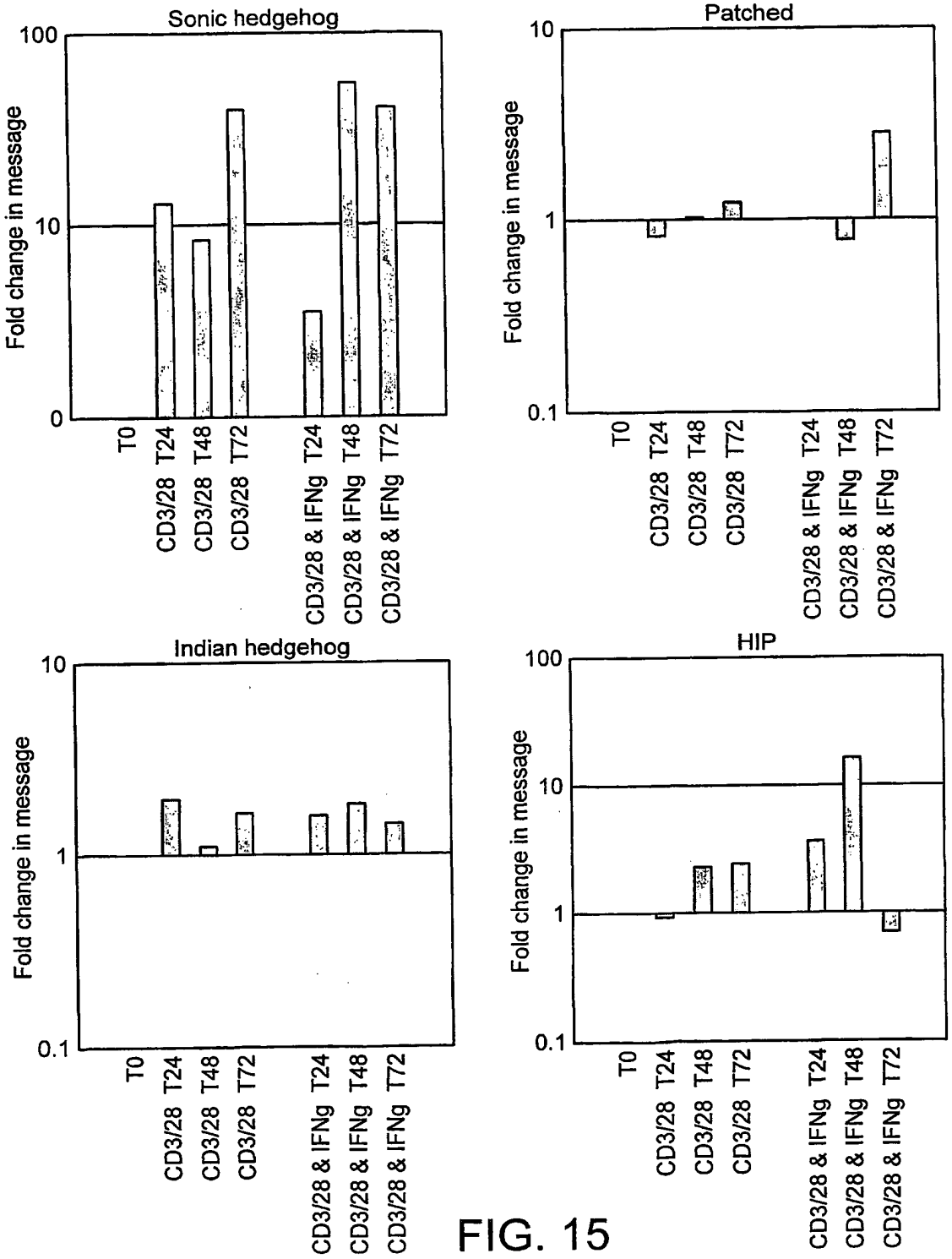
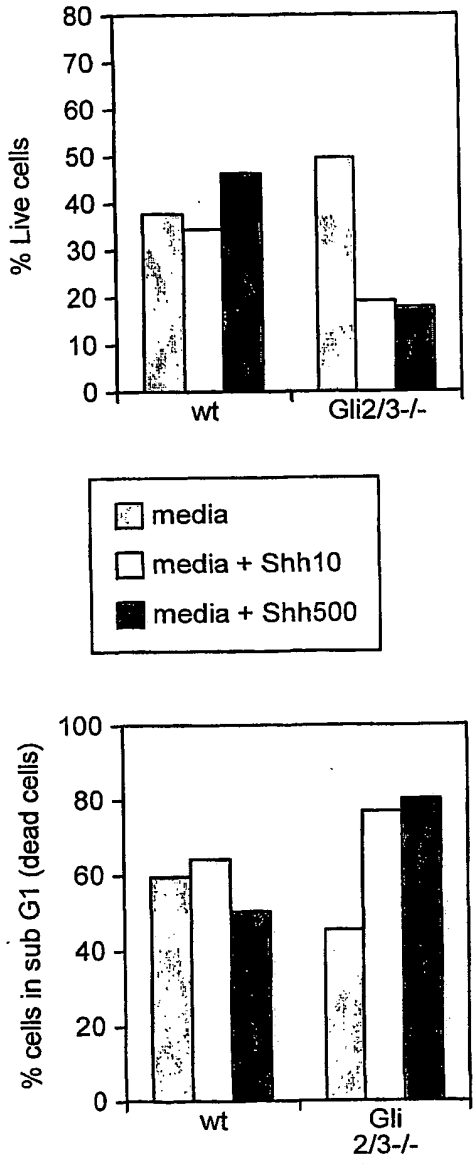


FIG. 15

Shh induced T cell survival requires Gli signalling



24h preculture +/- Shh + anti-CD3/28 for 72h

FIG. 16

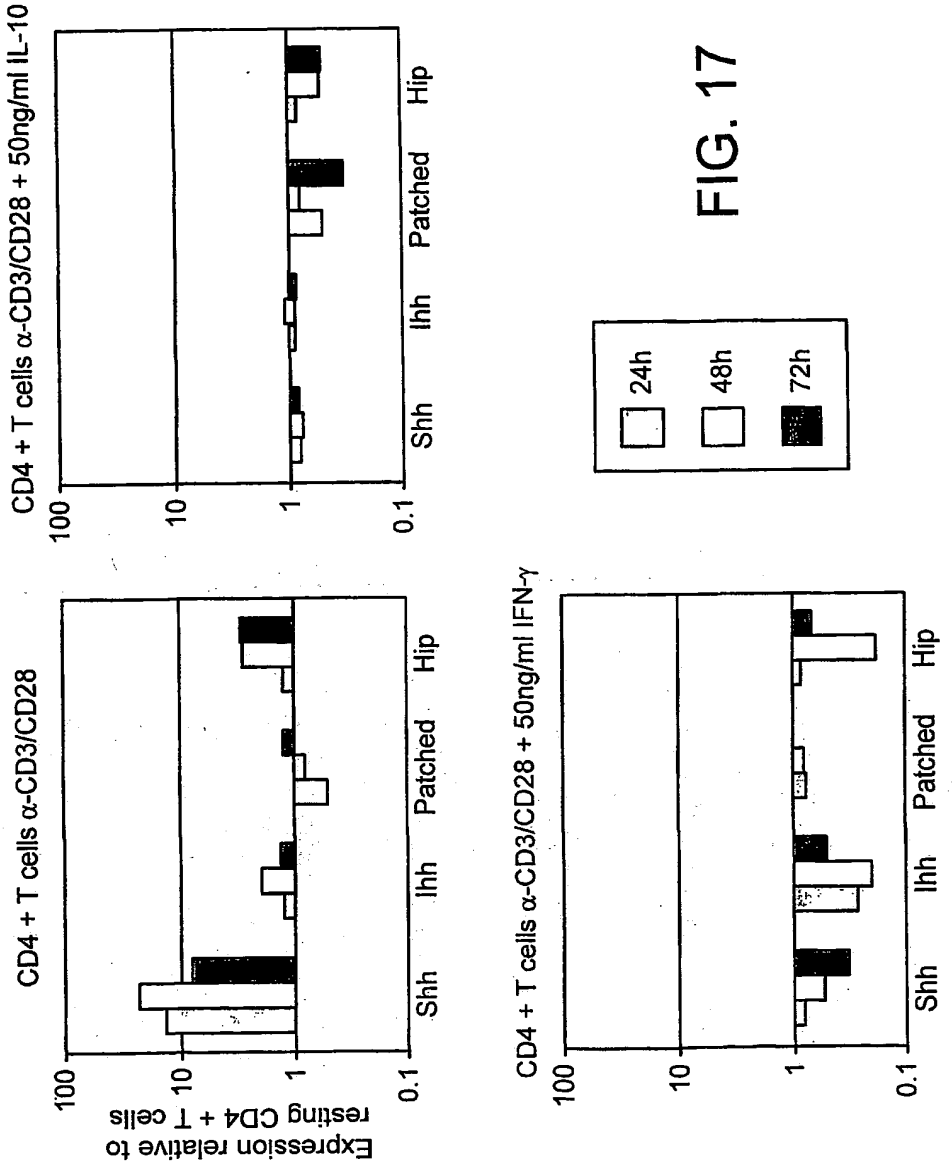

CD4 + T cells α-CD3/CD28 + 50ng/ml IL-10
CD4 + T cells α-CD3/CD28
CD4 + T cells α-CD3/CD28 + 50ng/ml IFN-γ
Expression relative to resting CD4 + T cells
100
10
1
0.1
Shh
Ihh
Patched
Hip
24h
48h
72h

FIG. 17

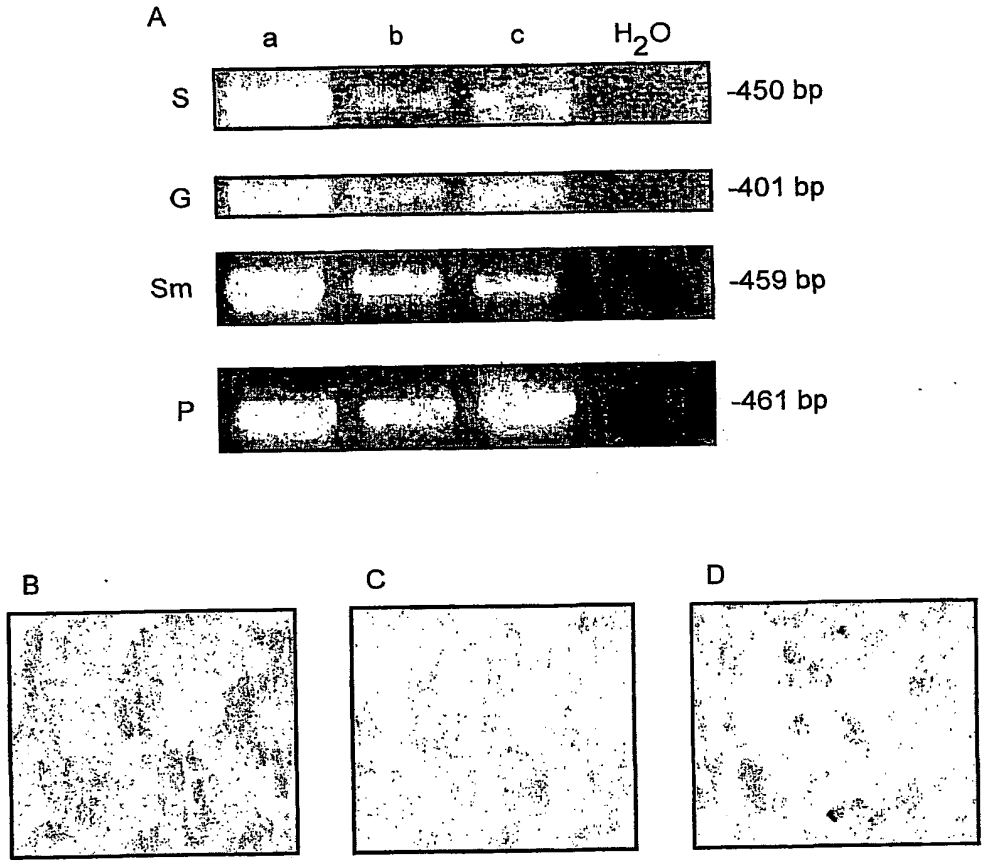


FIG. 18

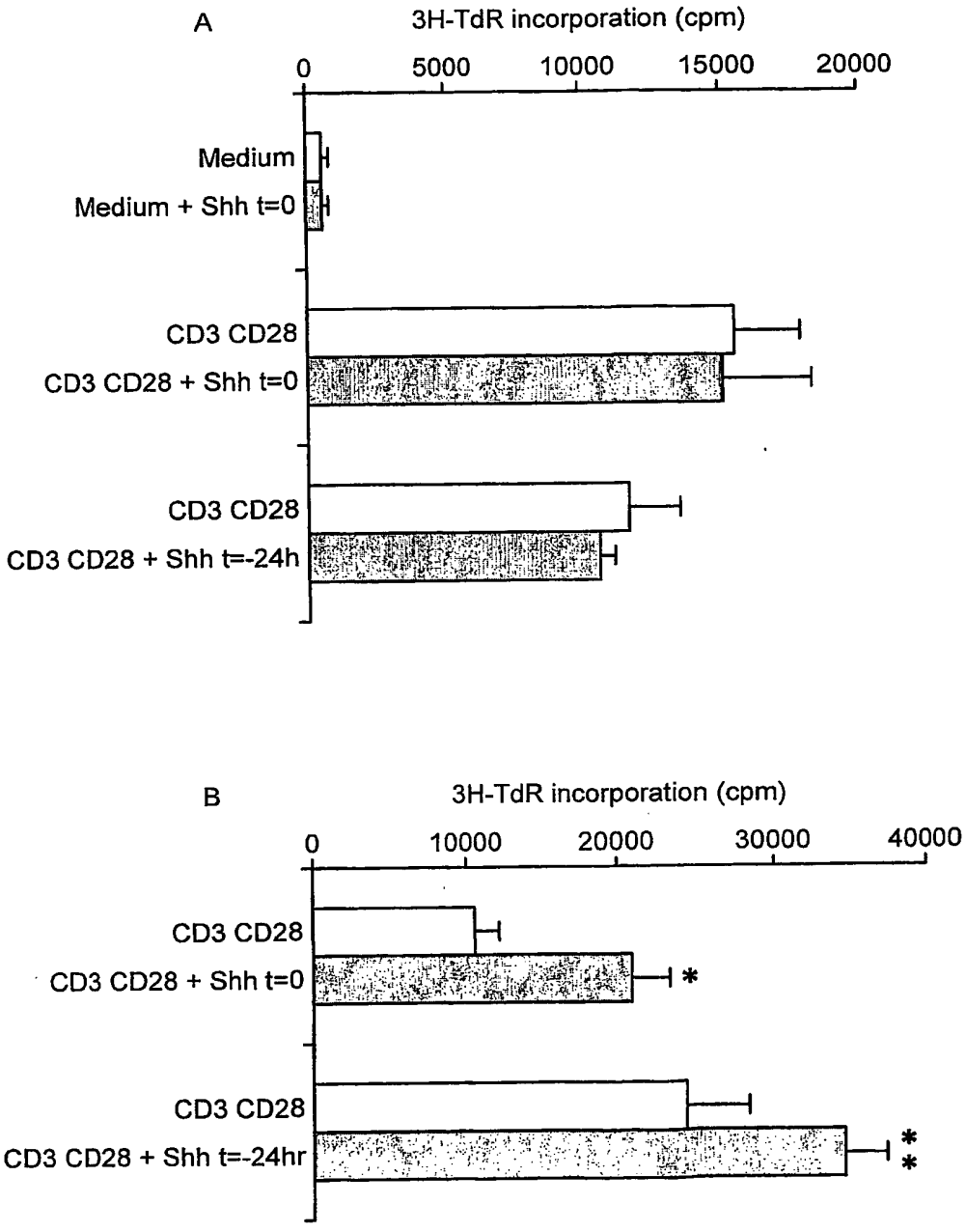
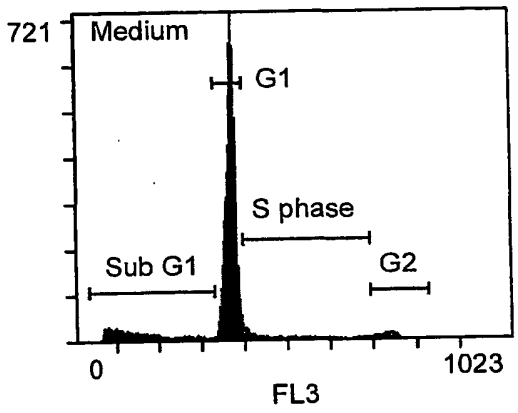
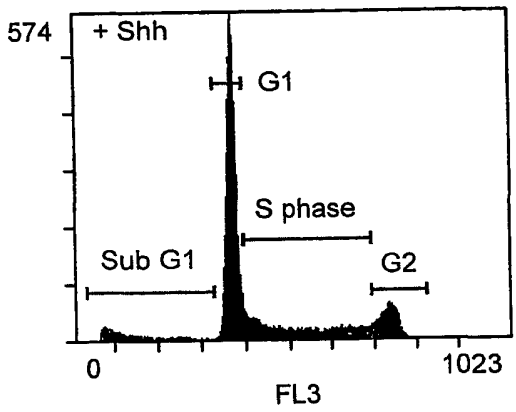

A
3H-TdR incorporation (cpm)
0
5000
10000
15000
20000
Medium
Medium + Shh t=0
CD3 CD28
CD3 CD28 + Shh t=0
CD3 CD28
CD3 CD28 + Shh t=-24h
B
3H-TdR incorporation (cpm)
0
10000
20000
30000
40000
CD3 CD28
CD3 CD28 + Shh t=0
*
CD3 CD28
CD3 CD28 + Shh t=-24hr
*
*

FIG. 19

A



B



C

Time in culture	Treatment	% live cells	% live cells in G1	% live cells in S/G2
24hr	Medium	74.9	98	2
	Shh	78.3	97	3
48hr	Medium	71.4	97	3
	Shh	73.1	97	3
72hr	Medium	73.5	96	4
	Shh	72.9	97	3

D

Exptl conditions	Treatment	% live cells	% live cells in G1	% live cells in S/G2	% increase in live cells in S/G2 with +Shh
Optimal anti-CD3/26	Medium	82.7	79.2	20.8	38.8%
	Shh at t=0	82.6	71.4	28.6	
Optimal anti-CD3/28	Medium	27.86	72.9	28.1	97.1%
	Shh at t=-24hr	30.02	44.6	55.4	
sub-optimal anti-CD3/28	Medium	50.9	78.3	21.7	61.7%
	Shh at 2=0	56.7	64.9	35.1	
sub-optimal anti-CD3/28	Medium	41.54	76.9	23.1	110%
	Shh at t=-24hr	64.71	51.5	48.5	

FIG. 20

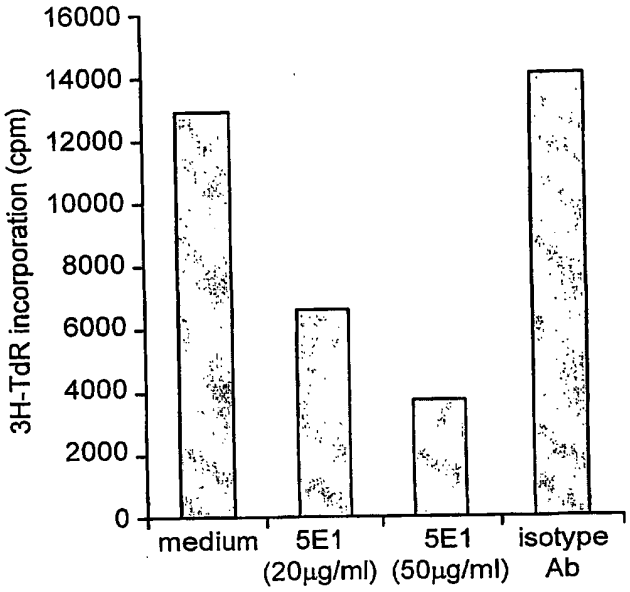


FIG. 21

Treatment	% live cells	% live cells in G1	% live cells in S/G2	% increase in live cells in S/G2 with +5E1
medium	50.94	78.3	21.7	NA
5E1 20µg/ml	50.12	86	14	35.5%
5E1 50µg/ml	47.91	96	4	81.6%
isotype control	54	82.4	17.6	NA

FIG. 24

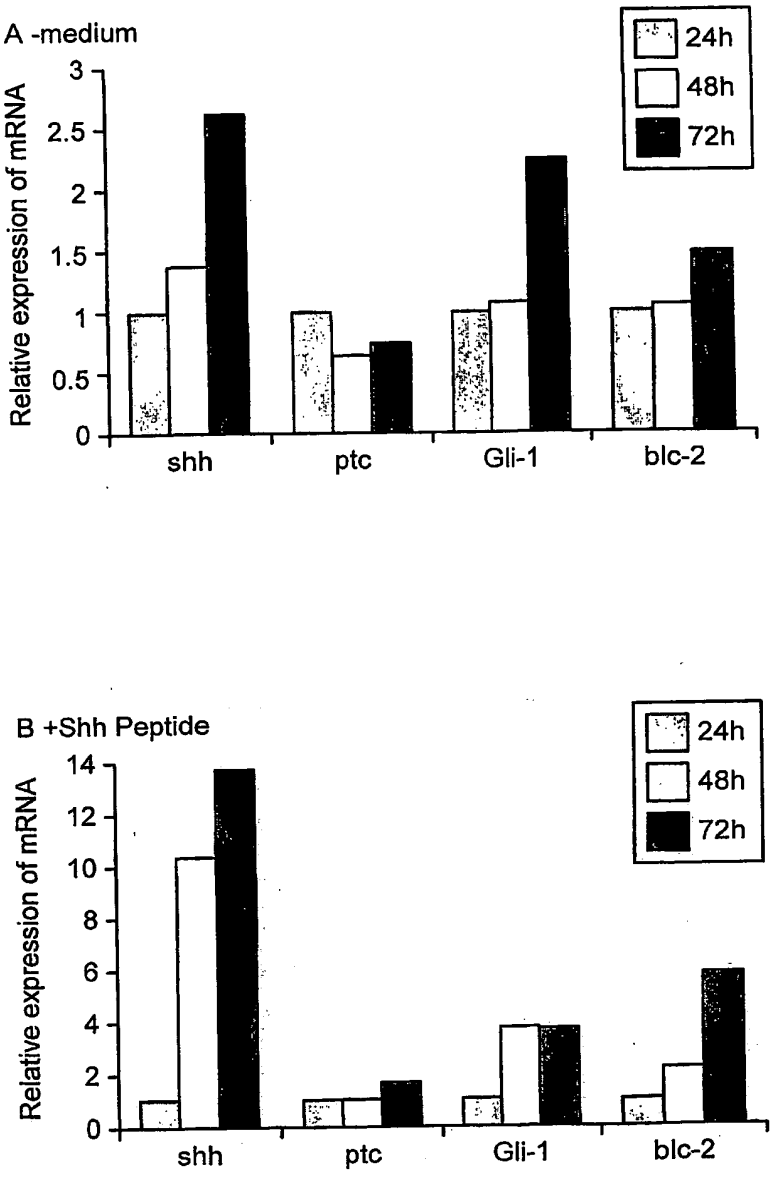

A -medium
Relative expression of mRNA
3
2.5
2
1.5
1
0.5
0
shh
ptc
Gli-1
blc-2
24h
48h
72h
B +Shh Peptide
Relative expression of mRNA
14
12
10
8
6
4
2
0
shh
ptc
Gli-1
blc-2
24h
48h
72h

FIG. 22

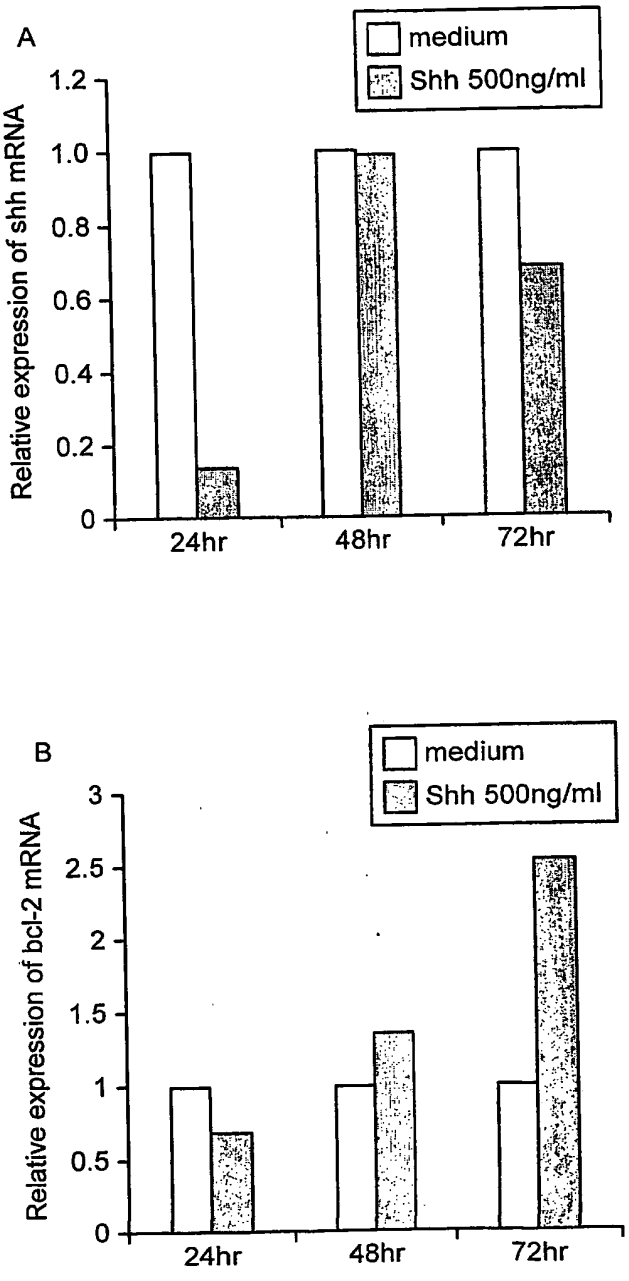

A
medium
Shh 500ng/ml
Relative expression of shh mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
24hr
48hr
72hr
B
medium
Shh 500ng/ml
Relative expression of bcl-2 mRNA
3
2.5
2
1.5
1
0.5
0
24hr
48hr
72hr

FIG. 23